Substance P effects on blood flow, fluid transport and vasoactive intestinal polypeptide release in the feline small intestine

I. Brunsson, J. Fahrenkrug*, M. Jodal, A. Sjöqvist and O. Lundgren

*Department of Physiology, Göteborg University, Gothenburg, Sweden and *Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark*

1. Substance P (SP) infusions were given close I.A. to the feline small intestine *in vivo* in a dose that produced plasma concentrations of 1–5 μM. This infusion regularly evoked a net fluid secretion measured with a gravimetric technique. Concomitantly, the release into blood of vasoactive intestinal polypeptide (VIP), a putative neurotransmitter of the enteric nervous system, increased.

2. The SP-induced fluid secretion was blocked by tetrodotoxin (7 μg close I.A.), a blocker of fast sodium channels in excitable tissues, and hexamethonium (10 mg (kg body wt)$^{-1}$, I.V.), a nicotinic receptor antagonist, suggesting that the SP effect was mediated by the enteric nervous system. In line with this it was shown that the SP-evoked release of VIP was also significantly diminished by hexamethonium.

3. Close I.A. infusions of methionine enkephalin (Met-enkephalin; 7–23 nmol min^{-1}) or electrical stimulation of the sympathetic nerve fibres (6 Hz) to the intestine markedly diminished net fluid secretion and the release of VIP caused by SP given close I.A.

4. The cyclo-oxygenase inhibitor diclofenac (5 mg (kg body wt)$^{-1}$, I.V.) or the histamine-1 receptor antagonist pyrilamine (10 mg (kg body wt)$^{-1}$, I.V.) did not influence the fluid secretion caused by SP, indicating that the effects of SP were not due to the actions of prostaglandins or histamine.

5. It is proposed that SP activates a nervous reflex arch that we have shown to be activated by various luminal stimuli, including cholera toxin. The proposed reflex is made up of at least three neurons: an afferent neuron going from the mucosa to the myenteric plexus, a cholinergic interneuron and a VIP-ergic neuron from the submucosal plexus controlling the enterocytes. Met-enkephalin and the sympathetic nerves decrease the fluid secretion by pre- or postsynaptic inhibition of reflex nervous activity.

We have previously shown in cat and rat experiments that the fluid secretion evoked by placing cholera toxin in the intestinal lumen or by exposing the intestinal serosa to hydrochloric acid is to a large extent mediated via an activation of reflexes in the enteric nervous system (Cassuto, Jodal, Tuttle & Lundgren, 1981; Sjöqvist, Cassuto, Jodal, Brunsson & Lundgren, 1982; Brunsson, Sjöqvist, Jodal & Lundgren, 1985; Brunsson, Fahrenkrug, Jodal, Sjöqvist, Theodorsson-Norheim & Lundgren, 1990). We noted that both types of secretory states were accompanied by an increased release of vasoactive intestinal polypeptide (VIP) into blood from the small intestine (Cassuto, Fahrenkrug, Jodal, Tuttle & Lundgren, 1981; Brunsson *et al.* 1990). The secretions were markedly diminished by blocking action potentials with tetrodotoxin (TTX) or by addition of a nicotinic ganglionic receptor antagonist. Furthermore, experimental evidence for the involvement of a tachykinin neuron was obtained in the experiments in which chemical peritonitis was induced by hydrochloric acid (Brunsson *et al.* 1990). The tachykinin neuron seemed to be more proximal than the cholinergic one in the reflex arch activated by acid, since the stimulated release of tachykinins was unaffected by hexamethonium. Finally, a significant correlation between change of VIP release and change of net fluid transport was shown (Brunsson *et al.* 1990).

Histochemical and electrophysiological studies show that substance P (SP) is present in nerve fibres throughout the intestinal wall and in nerve cell bodies in both the myenteric and the submucosal plexuses. SP activates enteric neurons and has been suggested to be one of the candidates responsible for the slow excitatory post-synaptic potentials (slow EPSPs) seen in both plexuses

(Furness & Costa, 1987). Moreover, SP induces, both *in vivo* and *in vitro*, an increase of transepithelial potential difference that is sensitive to TTX, implying that the effect is nerve mediated (Keast, Furness & Costa, 1985; Perdue, Galbraith & Davison, 1987; Greenwood, Doolittle, See, Koch, Dodds & Davison, 1990).

The observations briefly summarized above led Brunsson *et al.* 1985, 1990, to conclude that SP functions as a neurotransmitter in the afferent limb of the secretory reflex evoked by chemical peritonitis. The aim of the present study was to investigate where on the secretory reflex SP induced its effect on net fluid transport *in vivo*. Substance P was therefore infused close I.A. to investigate the effects of this peptide on net fluid transport and VIP release into blood in the cat small intestine. When administering substance P in amounts that evoked a net fluid secretion an increased VIP release was seen. A pharmacological analysis of this response was performed. Since the initial results indicated that prostaglandins may be involved in the SP-induced response, a more thorough investigation was performed to analyse this observation.

METHODS

The experiments were performed on cats of either sex which had been in our animal quarters for at least 1 week prior to the experiments. The cats showed no obvious signs of gastrointestinal disease and had been deprived of food for 24 h with free access to water.

Operative procedures

After induction with diethylether, the cats were anaesthetized with α-chloralose administered into the femoral vein (50 mg (kg body wt)$^{-1}$). A tracheotomy was made to assure free airways. An intravenous infusion of a glucose and bicarbonate solution (see below) was given at a rate of 5 ml h^{-1}. Such an infusion has been shown in earlier studies to maintain fluid and electrolyte balance and acid–base balance within normal limits (Haglund & Lundgren, 1972).

A mid-line abdominal incision was made and the greater omentum and spleen were extirpated. A segment of the jejunum was isolated with its vascular supply intact and was gently flushed with isotonic saline at body temperature. The rest of the jejunum, ileum and colon was extirpated together with the distal part of the duodenum and parts of the pancreas. The distal end of the remaining part of the duodenum was drained to the exterior by plastic tubing.

The splanchnic nerves were cut in order to minimize the extrinsic nervous influence on the function of the isolated intestinal segment. In some experiments the peripheral ends of the nerves were placed on silver electrodes for subsequent electrical stimulation. The vascular supply of the left adrenal gland was ligated and the right adrenal gland was denervated to diminish the influence of circulating catecholamines on intestinal function. A branch of the superior mesenteric artery was cannulated for local intra-arterial infusions. A femoral artery was cannulated and connected to a pressure transducer (Statham P23AC) for continuous recording of arterial blood pressure. In most experiments, atropine (0·5 mg (kg body wt)$^{-1}$) was given at the end of the operative procedure.

Measurement of net fluid transport

Net intestinal transepithelial fluid transport was measured by a gravimetric technique developed at our department and described in detail by Sjövall, Brunsson, Jodal & Lundgren (1983). In short, the intestinal segment was mounted on a specially designed plastic plate and covered by a thin plastic film to minimize evaporation from the tissue. The lumen of the distal end of the segment was connected to a small basin also situated on the plastic plate. This basin and the proximal end of the intestinal segment were connected by plastic tubing, the intestinal segment and tubing thereby creating a closed perfusion system. The luminal fluid was circulated by a roller pump at a constant rate of 1 ml min^{-1} in an aboral direction. A reservoir with a large volume (700 ml) was inserted into the closed perfusion system in order to minimize recirculation of the perfusate.

The total weight of the plastic plate with its basin and intestinal segment was continuously recorded by a force displacement transducer (Grass FT10C) and monitored on a Grass polygraph recorder. The recorded change in weight was interpreted as reflecting net fluid transport, an increase in weight reflecting net secretion. This method permits a continuous, on-line recording of net fluid transport. At the end of the experiment the intestinal segment was cut open and the serosal surface area was measured. Net fluid transport values were calculated in microlitres per minute and per 100 cm^2 serosal surface area.

Measurement of intestinal blood flow

After completing the operation, heparin was given I.V. (3 mg (kg body wt)$^{-1}$). The mesenteric vein was cannulated and the blood was diverted through a drop recorder unit and returned to the animal via the jugular veins. Intestinal blood flow was continuously monitored by the polygraph recorder and expressed in millilitres per minute and 100 g tissue, assuming an equal perfusion rate in the intestinal segment and perimesenteric lymphatic nodes (Lundgren & Wallentin, 1964). Mesenteric venous blood samples were collected for VIP concentration determinations from a three-way stopcock in the mesenteric catheter.

Experimental procedures

With the exception of the pyrilamine series, the experimental protocol was the same for all experiments in which drugs were administered. Firstly, a control period of at least 30 min of steady-state net fluid transport was recorded. Substance P (SP), dissolved in physiological saline with 1% albumin, was then infused close I.A. for 30 min at a rate of 2–20 μg min^{-1}. The dose was chosen to evoke a net fluid secretion. After a 15 min period of constant fluid secretion the test substance was injected. After another 15 min the SP infusion was stopped and the recorded variables were followed for another 15–30 min.

Two major types of experiments were performed. In one type only fluid transport and blood flow were followed, whereas in the other type blood samples were also taken for determination of intestinal release of VIP. Three experimental series of the first type were performed.

Tetrodotoxin experiments ($n = 5$). Tetrodotoxin (TTX, 7 μg) was given close I.A. while occluding the superior mesenteric artery for ~2 min. Before the blood flow was restored the

vasculature of the intestinal segment was flushed with 5 ml of saline to avoid systemic effects of TTX. The efficacy of the nervous blockade was tested by stimulating electrically (see below) the splanchnic nerves for 0·5–1 min before and repeatedly after giving the drug. The effect of TTX was evaluated by the magnitude of the vasoconstrictor response.

Pyrilamine experiments ($n = 3$). Pyrilamine, a H_1-receptor antagonist, was administered I.V. at a dose of 10 mg (kg body wt)$^{-1}$, which abolished the increase of intestinal blood flow recorded after addition of 1 μg histamine close I.A. The drug was given prior to the I.A. infusion of SP.

Diclofenac experiments ($n = 4$). Diclofenac, an inhibitor of the cyclo-oxygenase pathway in the prostanoid cascade, was given I.V. at a dose of 5 mg (kg body wt)$^{-1}$ during the I.A. infusion of SP.

Venous blood samples were collected for VIP determinations in the following four series of experiments.

Stimulation of the regional sympathetic fibres ($n = 6$). In one type of experiment SP was infused close I.A. for 15 min during the continuous stimulation of the regional sympathetic fibres. Bilateral stimulation of the nerves was performed via ring electrodes. The stimulation parameters were set at 5 V, 5 ms and 6 Hz. The results from such experiments were compared with those obtained during a 15 min period of SP infusion alone.

Hexamethonium experiments ($n = 6$). Hexamethonium (10 mg (kg body wt)$^{-1}$), a nicotinic receptor blocker, was given I.V.

Indomethacin experiments ($n = 5$). Indomethacin (7 mg (kg body wt)$^{-1}$) was injected I.V.

Methionine enkephalin experiments ($n = 5$). Methionine enkephalin (Met-enkephalin) was infused via a second arterial branch of the superior mesenteric artery at a rate of 7–23 nmol min^{-1} during the second half of a 30 min period of close I.A. infusion of SP. The dose was chosen to inhibit the fluid secretion caused by SP.

Analyses of the VIP concentration in plasma

Blood samples for VIP analyses were taken from the mesenteric vein and femoral artery. Venous samples were collected during control conditions and at 5 and 10 min during each experimental procedure. Three arterial samples were taken at regular intervals during each experiment.

Blood (2 ml) was collected directly into ice-chilled plastic tubes containing 0·1 ml aprotinin (a protease inhibitor) and immediately centrifuged at +4 °C. One millilitre plasma was transferred to another test-tube and was kept frozen at −20 °C until analysed. The blood cells were returned to the animal and 1·5 ml of a 4% albumin solution was added for each sample taken. VIP concentrations were determined by radioimmunoassay (RIA). The VIP-RIA has been described in detail by Fahrenkrug & Schaffalitzky de Muckadell (1977).

Calculations of peptide release

The arteriovenous plasma concentration difference multiplied by the intestinal plasma flow was taken as the net peptide release into blood and expressed in picomoles per minute and per 100 g intestinal tissue. A mean release from the two measurements made during control conditions as well as a mean value of the release at 5 and 10 min were calculated.

Solutions and drugs

The intestinal lumen was exposed to a modified Krebs–Henseleit solution with the following composition (mM): 122 NaCl, 4·7 KCl, 1·2 KH_2PO_4, 1·2 $MgCl_2.6H_2O$, 2·5 $CaCl_2$, 25 $NaHCO_3$ and 30 mannitol. The total osmolality of the solution was 305–315 mosmol (kg H_2O)$^{-1}$. The continuous I.V. infusion contained (mM): 100 $NaHCO_3$ and 278 glucose.

The following drugs were used: aprotinin (Trasylol®, Bayer AG, Leverkusen, Germany), atropine sulphate, bovine albumin, hexamethonium bromide, methionine enkephalin, pyrilamine, prostaglandin E_2, substance P, tetrodotoxin (all Sigma Chemicals, St Louis, MO, USA), diclofenac (Voltaren®, Geigy, Basel, Switzerland) and indomethacin (Confortide®, Dumex AB, Sweden).

Statistics

Wilcoxon's signed rank test or the sign test was used. A P value of 0·05 or less was considered statistically significant. Values are given as means ± S.E.M. Student's t test was used to test regression lines and correlations.

RESULTS

Studies of intestinal blood flow and fluid transport

Substance P (SP) was infused close I.A. at a rate of 2–20 μg min^{-1} (1·5–15 nmol min^{-1}), corresponding to an increase of the arterial plasma concentration of 1–5 μM. The peptide infusion evoked, in some experiments, a transient increase of blood flow. The flow values given in Tables 1 and 2 represent the peak values observed. The SP infusion caused a stable secretory response (Tables 1 and 2; Fig. 1), which did not exhibit tachyphylaxis and which was not influenced by the muscarinic receptor antagonist atropine (0·5 mg (kg body wt)$^{-1}$; data not shown). Fluid secretion ceased within 5 min after stopping the infusion.

The secretory response to SP was markedly diminished by giving the sodium channel blocker TTX (7 μg close I.A.; Fig. 1), whereas giving H_1 receptor blocker pyrilamine (10 mg (kg body wt)$^{-1}$, I.V.) prior to the SP infusion in three experiments did not significantly influence the SP effect (data not shown). The SP-induced secretion was not affected by I.V. administration of diclofenac (5 mg (kg body wt)$^{-1}$), an inhibitor of prostaglandin synthesis (Fig. 1). Arterial pressure was increased by diclofenac from 111 ± 6 to 135 ± 7 mmHg ($n = 4$), while the blood flow change induced by SP was not significantly influenced by any of the drugs.

Studies of intestinal blood flow, net fluid transport and VIP release

In an initial series of experiments the effects of close I.A. infusions of SP on VIP release and fluid transport was studied in five experiments. The SP infusion turned fluid absorption (62 ± 33 μl min^{-1} (100 cm)$^{-2}$) into net fluid

Table 1. The effect of sympathetic stimulation on the substance P-evoked changes of arterial blood pressure (BP; mmHg), intestinal blood flow (BF; ml min^{-1} (100 g)$^{-1}$), intestinal net fluid transport (NFT; μl min^{-1} (100 cm)$^{-2}$) and intestinal release of vasoactive intestinal polypeptide (VIP; pmol min^{-1} (100 g)$^{-1}$)

	Control	SP	Symp + SP
BP	119 ± 61	121 ± 8	141 ± 10
BF	41 ± 4	43 ± 8	53 ± 2
NFT	77 ± 46	−284 ± 68 [a]	21 ± 53 [b]
VIP release	1·12 ± 0·26	4·21 ± 1·21 [a]	2·40 ± 1·23 [b]

Values are means ± S.E.M. Number of observations, 6. Minus sign denotes net fluid secretion. [a]Statistically significant from control; [b]statistically significant from SP.

secretion (244 ± 45 μl min^{-1} (100 cm)$^{-2}$; $P < 0·05$). Concomitantly, VIP release into intestinal venous blood increased from 1·11 ± 0·16 to 4·17 ± 0·80 pmol min^{-1} (100 g)$^{-1}$ ($P < 0·05$). Tables 1 and 2 summarize further experiments in which VIP release was studied simultaneously with intestinal fluid transport and blood flow.

Effect of splanchnic nerve stimulation

In six experiments SP was infused before and during a concomitant stimulation of the sympathetic nerves to the gut (6 Hz, 5 ms, 5 V). In contrast to the effect seen during control conditions, infusing SP close I.A. during a nervous stimulation did not evoke any net fluid secretion. Furthermore, the SP-induced VIP release was attenuated (Table 1).

Hexamethonium experiments

Administration of the nicotinic receptor antagonist hexamethonium (10 mg (kg body wt)$^{-1}$, I.V.) turned the SP-induced fluid secretion into absorption and, concomitantly, the augmented VIP release was abolished. Intestinal blood flow decreased since arterial pressure was lowered by the drug (Table 2).

Met-enkephalin experiments

The effects of Met-enkephalin (7–23 nmol min^{-1} infused close I.A.) on the SP-induced changes were similar to those

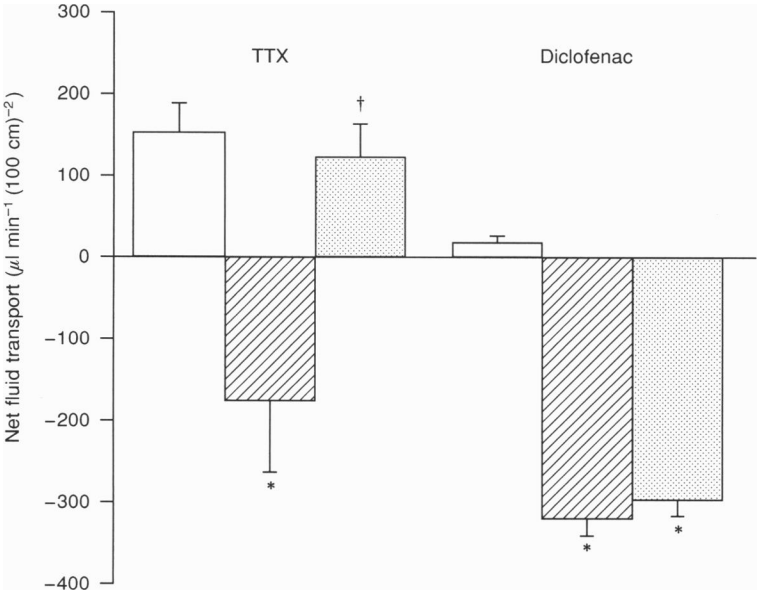

Figure 1. The effects of tetrodotoxin (TTX; 5 observations) and diclofenac (4 observations) on the net fluid secretion in the feline small intestine evoked by close I.A. infusion of substance P (SP)

The open columns illustrate net fluid transport during control conditions; the hatched and shaded columns depict fluid transport during infusions of SP before and after giving drug, respectively. Values are means ± S.E.M. Negative values indicate net fluid secretion into the intestinal lumen. * and † indicate statistically significant differences compared with control and SP infusion, respectively.

Table 2. The effect of hexamethonium (10 mg (kg body wt)$^{-1}$), Met-enkephalin (7–23 nmol min^{-1}) and indomethacin (7 mg (kg body wt)$^{-1}$) on the substance P (SP)-evoked changes of arterial blood pressure (BP; mmHg), intestinal blood flow (BF; ml min^{-1} (100 g)$^{-1}$), intestinal net fluid transport (NFT; μl min^{-1} (100 cm)$^{-2}$) and intestinal release of vasoactive intestinal polypeptide (VIP; pmol min^{-1} (100 g)$^{-1}$)

	Control	SP	SP + drug	Drug
Hexamethonium ($n = 5$)				
BP	112 ± 6	119 ± 8	87 ± 9 [a,b]	89 ± 10 [a,b]
BF	38 ± 5	41 ± 6	25 ± 1 [a,b]	22 ± 1 [a,b]
NFT	48 ± 55	−232 ± 82 [a]	146 ± 47 [b]	185 ± 42 [b]
VIP release	0·79 ± 0·25	1·84 ± 0·44 [a]	0·33 ± 0·13 [b]	0·18 ± 0·06 [a,b]
Met-enkephalin ($n = 5$)				
BP	123 ± 11	121 ± 10	112 ± 7	110 ± 4
BF	30 ± 5	32 ± 4	36 ± 7	37 ± 7
NFT	84 ± 63	−195 ± 71 [a]	12 ± 99 [b]	120 ± 55 [b]
VIP release	1·07 ± 0·31	2·51 ± 0·97 [a]	0·72 ± 0·35 [b]	0·25 ± 0·14 [a,b]
Indomethacin ($n = 5$)				
BP	115 ± 5	109 ± 7	90 ± 8	102 ± 7
BF	35 ± 4	41 ± 5	34 ± 3	39 ± 4
NFT	111 ± 51	−180 ± 65 [a]	115 ± 65 [b]	164 ± 54 [b]
VIP release	0·82 ± 0·18	4·07 ± 1·63 [a]	0·51 ± 0·30 [b]	0·062 ± 0·077 [a,b]

Values are means ± S.E.M.; n = number of observations. Negative values denote net fluid secretion. [a]Statistically significant from control; [b]statistically significant from SP.

observed after giving hexamethonium, although blood flow was not influenced since arterial pressure remained constant (Table 2).

Indomethacin experiments

The effects of the cyclo-oxygenase inhibitor indomethacin (7 mg kg^{-1}, I.V.) on the SP-induced changes were studied in five experiments. The results are summarized in Table 2. It is apparent that indomethacin abolished the effects of SP on net fluid transport and rate of VIP release. No effect on intestinal blood flow was seen.

Correlation between changes of fluid transport and VIP release

A significant correlation between change of net fluid transport and change of VIP release was shown in all series of experiments, implying that a decreased fluid uptake or an increased fluid secretion was accompanied by an increase in VIP release. The correlation coefficient varied between 0·64 and 0·74 in individual experiments. Pooling all the results ($n = 28$) also demonstrated a significant correlation between the two variables, the correlation coefficient amounting to 0·66 ($P < 0·001$). No significant correlation could be demonstrated between changes in intestinal blood flow and VIP release.

DISCUSSION

Intra-arterial infusions of SP in the cat in doses resulting in plasma concentrations of 1–5 μM always induced a net fluid secretion that was accompanied by an increased VIP release into the venous effluent. Giving TTX close I.A. totally abolished the secretory effect of SP. Similar results have also been reported by Keast *et al.* (1985) and Perdue *et al.* (1987) in guinea-pig *in vitro*, and by Greenwood *et al.* (1990) in ferret *in vivo*. These observations strongly suggest that SP elicits its secretory effect mainly via nerves. The effect of hexamethonium (Table 2) also supports this conclusion. Finally, the lack of effect of pyrilamine suggests that SP, at the dose used, did not evoke its effects through a release of histamine from mast cells.

The SP-induced fluid secretion in the cat was blocked by indomethacin but not by diclofenac, a rather specific cyclo-oxygenase blocker. This indicates that at least part of the indomethacin effect is unspecific. Similar observations have been made by others. Thus Northover (1977) emphasized that indomethacin also acts as a calcium antagonist. The results of Katz & Creasy (1981), Edlund *et al.* (1985) and Jansson (1990) show that the drug also possesses vasoconstrictor properties. Furthermore, Gustafsson & Delbro (1993) found that indomethacin

elicits a motility response in the small intestine. None of these effects was evoked by diclofenac. It is thus obvious that effects obtained with indomethacin, at least in the concentrations usually used *in vivo*, have to be interpreted with care.

If prostaglandins participated in the SP-induced effects reported in this study, at least two mechanisms seem possible. Firstly, SP may stimulate prostaglandin release which, in turn, activates the postsynaptic neuron. A SP induction of prostaglandin release has been reported from, for example, macrophages (Hartung, Wolters & Toyka, 1986) and muscle cells (Yousufzai, Akhtar & Abdel-Latif, 1986). Furthermore, PGE_2 has been shown to cause intestinal secretion via nerves in the cat jejunum *in vivo* (Brunsson, Sjöqvist, Jodal & Lundgren, 1987) and in rat colon descendens *in vitro* (Diener, Bridges, Knobloch & Rummel, 1988). The second possibility is that endogenously released prostaglandins in some way modulate the membrane of the postsynaptic neuron, resulting in a potentiation of the SP stimuli.

Several locations seem possible for the SP action on the secretory nerves of ENS. One is at the ganglionic level in the submucosal and/or myenteric plexuses. Electrophysiological studies show that SP can elicit changes in the membrane potential of many myenteric and submucosal neurons that mimic in several respects the so-called slow excitatory postsynaptic potentials (EPSPs; Furness & Costa, 1987; Wood, 1989; Mihara, 1993). Furthermore, in Ussing chamber studies of intestinal electrolyte secretion *in vitro*, using segments stripped of their muscle layer, SP induces a secretion which is partly inhibited by atropine (Keast *et al.* 1985; Mathison & Davison, 1989). This indicates that SP can induce secretory responses via an activation of submucosal neurons which are known to express SP receptors (neurokinin-1 receptors; Reddix & Cooke, 1992).

A submucosal site of action for SP seems less likely in the present study, since hexamethonium blocked both the SP-induced secretion and the VIP release. This clearly indicates that SP does not act on the efferent neuron in

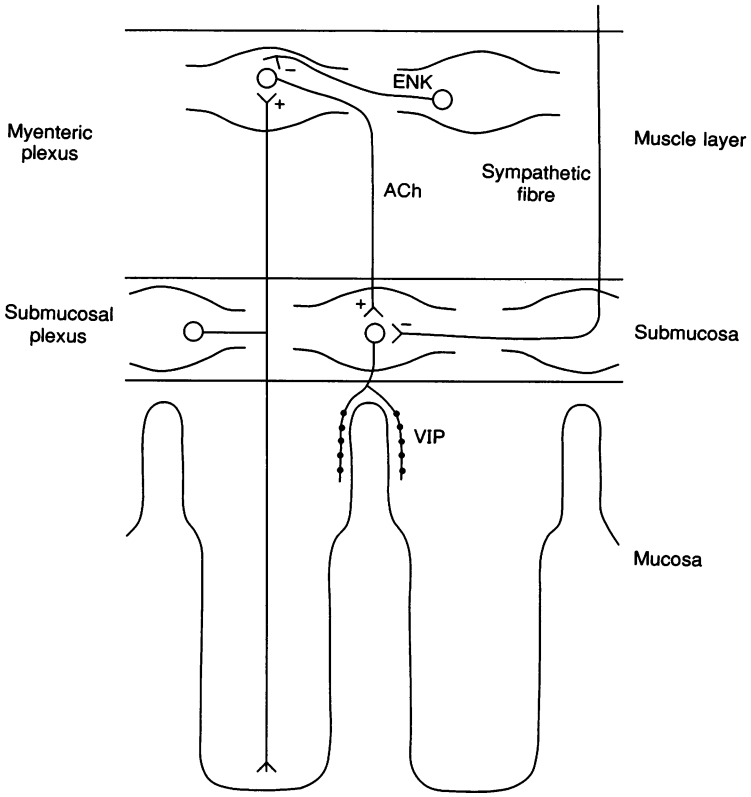

Figure 2. Schematic illustration of one possible interpretation of the results of the present study

Luminal stimuli, such as cholera toxin, activate a secretory nervous reflex, which consists of three neurons: an afferent neuron with unknown neurotransmitter, a cholinergic interneuron (ACh) with its cell body in the myenteric plexus and a VIP-ergic neuron in the submucosa influencing the intestinal epithelium. We propose that the infused SP influences epithelial function mainly via SP-receptors located on the cholinergic interneuron. The sympathetic nervous influence on the secretory reflex is localized to the submucosal plexus, but a myenteric point of action is also possible. The effect of Met-enkephalin observed in this study is proposed to mimic the action of an inhibitory enkephalinergic neuron located in the myenteric plexus.

the submucosal plexus, but favours an SP-induced activation of a cholinergic interneuron or of neurons still more 'proximal' in an intramural secretory reflex. This proposal is supported by the observation that SP does not induce any release of acetylcholine from neurons in the submucosal plexus (Yau & Youther, 1982; Yau, Dorsett & Youther, 1990), indicating a lack of SP receptors on the cholinergic neurons of the submucosa.

The proposal that the SP secretory effect in the present study is localized to the myenteric plexus or to a more 'proximal' site in an intramural reflex arch is not compatible with the results of the *in vitro* studies, which indicate that SP acts via nerves also in preparations devoid of the myenteric plexus (guinea-pig: Keast *et al.* 1985; Perdue *et al.* 1987; ferret: Greenwood *et al.* 1990). This difference could be explained by the achievement of higher tissue concentrations of SP at the submucosal plexus *in vitro* than *in vivo*. It could also be explained by species differences.

An SP effect mainly on the myenteric plexus is also suggested by the results of the Met-enkephalin experiments (Table 2). Met-enkephalin markedly attenuated the SP-evoked secretion, although Met-enkephalin does not induce any hyperpolarization of the submucosal neurons (Surprenant & North, 1985). On the other hand, Met-enkephalin hyperpolarizes enteric neurons in the myenteric plexus (North, 1982; Wood, 1989). Finally, Gaginella, Rimele & Wietecha (1983) failed to demonstrate any opiate receptor on enterocytes, which excludes any direct epithelial action of Met-enkephalin. All these observations indicate that Met-enkephalin inhibits the fluid secretion evoked by SP mainly via receptors located on myenteric neurons.

To summarize the discussion concerning the site of action of SP with regard to fluid transport, the available experimental evidence suggests an action mainly on the myenteric plexus.

The observations made during sympathetic nerve stimulation do not argue against a myenteric site of action for SP. The sympathetic innervation is dense in both of the two major plexuses in the intestinal wall. Two sites of action have been demonstrated for the sympathetic adrenergic nerve fibres. Several studies indicate that the adrenergic influence may cause a presynaptic inhibition of the release of acetylcholine in enteric synapses in both plexuses (Nishi & North, 1973; Hirst & Silinsky, 1975; North, 1982; Wood, 1989). Furthermore, adrenergic nerves are known to evoke slow inhibitory postsynaptic potentials (IPSPs) in both myenteric and submucosal neurons (Furness & Costa, 1987; Wood, 1989; Mihara, 1993). In the submucosal plexus the effect is only on the non-cholinergic neurons controlling the epithelium (Bornstein, Costa, Furness & Lang, 1986). Our findings can thus be explained by a pre- and/or postsynaptic action of the sympathetic fibres.

The findings of this study are in agreement with our earlier studies on the involvement of the enteric nervous system in different secretory states in the small intestine, including cholera. Based on these studies we have proposed a model (Fig. 2; see e.g. Lundgren, Svanvik & Jivegård, 1989) for the organization of intestinal secretory reflexes consisting of at least three neurons: an afferent neuron with its synapse in the myenteric plexus, an efferent VIP neuron with its cell soma in the submucosal plexus and a cholinergic interneuron connecting the myenteric plexus with the submucosal one. We propose that SP infused close I.A. mainly activates the two 'efferent' neurons of the secretory reflex via receptors located on the cholinergic interneuron (Fig. 2). As discussed above, it seems less likely that SP acts on the submucosal plexus. In agreement with the discussion above, the sympathetic and enkephalinergic inhibitory neurons are localized to the submucosal and the myenteric plexuses, respectively (Fig. 2). The inhibitory effect may be located pre- and/or postsynaptically.

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